Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1737–1740

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Isoindolines: A New Series of Potent and Selective Endothelin-A Receptor Antagonists

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Received 15 February 2001; accepted 20 April 2001

Abstract—1,3-Disubstituted isoindolines have been discovered as a new class of potent functional ET_A selective receptor antagonists through pharmacophore analysis of existing nonpeptide endothelin antagonists. The structure–activity relationships for both the *trans* and the *cis* series of isoindolines are discussed.

Endothelins (ET-1, ET-2, and ET-3) are a family of potent vasoconstrictive and mitogenetic peptides, consisting of 21-amino acid residues originally isolated from conditioned medium of vascular endothelial cells.[1,2] These peptides are known to elicit a number of biological effects contributing to cardiovascular and renal dysfunctions[3] through interaction with specific G-protein coupled receptors. Two human receptor subtypes, ET_A and ET_B, have been fully characterized.[4,5] The ET_A receptor subtype is selective for ET-1 and ET-2 over ET-3, and is predominantly located in vascular smooth muscle, where it mediates vasoconstriction and smooth muscle proliferation.[6] The ET_B subtype is non-selective and binds all three isopeptides with nearly equal affinity, and presumably mediates either vasodilation or vasoconstriction, depending upon the tissue type.[7,8] Pharmacological studies have suggested that endothelins play a role in the pathophysiology of a large number of diseases including asthma,[9] coronary vasospasm, myocardial infarction,[10] pulmonary hypertension,[11] restenosis,[12] and atherosclerosis.[13] Furthermore, BQ 123, a peptidic ET_A selective receptor antagonist, has been shown to be efficacious in in vivo disease models, most notably hypertension[14] and acute renal failure.[15] Thus, several research groups have been intensely involved in developing ET receptor antagonists in order to evaluate their viability as therapeutic agents.

There have been numerous reports of nonpeptide ET receptor antagonists with a wide range of affinity for the ET_A and the ET_B receptor subtypes [e.g., SB-209670 (**1**),[16] L-749,329 (**2**)[17] and PD-156707 (**3**)].[18] As illustrated in Figure 1, the common pharmacophore among these structures appears to be an acidic functional group between three appropriately positioned aromatic substituents. Compound **1**, an indane dicarboxylic acid analogue, has been described as one of the most potent dual ET_A/ET_B receptor antagonists, K_i (ET_A) = 0.43 nM and K_i (ET_B) = 14.7 nM.[16] Thus, we envisioned that substitution of the indane ring system with an isoindoline bearing an acidic group attached to the nitrogen

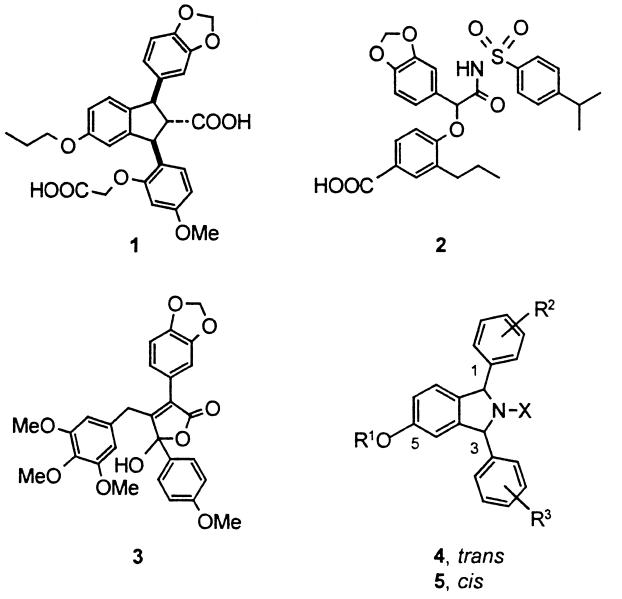

Figure 1. Nonpeptide endothelin antagonists.

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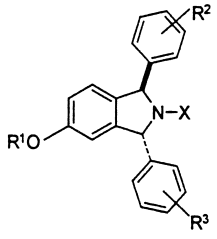
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00273-6

atom, compounds **4** and **5**, should lead to a new class of ET antagonists with high affinity for both receptors. 1,3-Disubstituted isoindolines, missing the chiral center at the 2-position, should be synthetically more accessible than indane 2-carboxylic acid analogues. Furthermore, introduction of a basic nitrogen to the backbone of the template might result in compounds with improved bioavailability. To test our hypothesis we prepared a variety of *trans* and *cis* 1,3-disubstituted isoindoline analogues **4** and **5**, respectively. We selected to retain an alkoxy substituent at the 5-position and 3,4-(methylenedioxy)phenyl group at the 3-position of the isoindoline ring system analogously to compound **1**. Most of the compounds **4** and **5** are acetic acid analogues at the isoindoline nitrogen atom.

We have previously reported a convenient, regio- and stereoselective synthesis for the efficient preparation of highly functionalized 1,3-disubstituted isoindoline analogues.[19] A variety of derivatives were prepared using the described protocols combined with methods widely known in the literature.[20] Compounds **4** and **5** were then evaluated for in vitro inhibition of [^{125}I]ET-1 binding to membrane preparations of CHO cells expressing the human ET_A or ET_B receptor subtype.[21] Surprisingly, compounds **4** and **5** were both identified as selective ET_A receptor antagonists. Only compound **5a** (Table 2) exhibited moderate, submicromolar binding affinity for the ET_B receptor (ET_A–IC_{50} = 4.1 nM; ET_B–IC_{50} = 770 nM).[22] The oxamic acid derivative **4f** (Table 1; ET_A–IC_{50} = 12 nM) was further shown to antagonize the ET-1 induced contraction of rat aorta with a pK_B value of 7.7.[23]

In general, the corresponding *trans* and *cis* isomers are equally potent ET_A receptor antagonists. However, the *cis* series of compounds appears to be more sensitive to modifications, especially at the isoindoline nitrogen. As the examples in Tables 1 and 2 illustrate, the second carboxylic acid in the aryl substituent at the isoindoline 3-position is essential for the binding activity in both *trans* and *cis* series of compounds. For example, analogues **4a** (ET_A–IC_{50} = 5.4 nM) and **5a** are about 20 times more potent than the corresponding monocarboxylic acids **4b** and **5b**. The importance of the second carboxylic acid group for the binding affinity is further demonstrated with compound **4k** in which the side-chain acid is replaced with a hydroxymethyl group. This modification totally abolished the inhibitory activity (ET_A–IC_{50} > 10 μM). In the *trans* series of compounds the acetic acid moiety bound to the isoindoline nitrogen atom can be substituted with other acidic groups such as an oxoacetic acid, analogue **4f**, and a tetrazole, analogue **4g** (ET_A–IC_{50} = 31 nM). Replacement of the side-chain acid with a tetrazole, compound **4h**, or increased distance between the two acids, compound **4i**, resulted in about 6- and 4-fold decrease in the potency, respectively. However, in the case of *cis* isoindolines these modifications are not well tolerated (e.g., the two tetrazole containing derivatives, compounds **5e** and **5k**, and the oxamic acid analogue **5f** have IC_{50} values greater than 10 μM). On the contrary, introduction of a geminal dimethyl group to the α-position of the side-chain carboxylic acid, compounds **4j** (ET_A–IC_{50} = 68 nM) and **5d** (ET_A–IC_{50} = 14 nM), was better tolerated by the *cis* isomer. In the monocarboxylic acid series the trimethoxyphenyl analogue **4o** (ET_A–IC_{50} = 66 nM) has

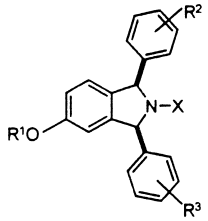
Table 1. Inhibition of [^{125}I]ET-1 binding to ET_A and ET_B receptors by *trans* 1,3-disubstituted isoindoline analogues **4**



Compd	R^1	X	R^2	R^3	ET_A–IC_{50} (nM)[a]	ET_B–%Inh @ 1 μM
4a	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 4-methoxy	5.4±0.2	15
4b	Et	CH_2COOH	3,4-Methylenedioxy	4-Methoxy	96±21	9
4c	Et	CH_2COOH	3,4-Methylenedioxy	2-Hydroxy; 4-methoxy	77±27	4
4d	*n*-Pr	CH_2COOH	3,4-Methylenedioxy	4-Methoxy	120±25	nd[b]
4e	Me	CH_2COOH	3,4-Methylenedioxy	4-Methoxy	3300±430	1
4f	Et	COCOOH	3,4-Methylenedioxy	2-OCH_2COOH; 4-methoxy	12±4.9	11
4g	Et	5-Tetrazolyl	3,4-Methylenedioxy	2-OCH_2COOH; 4-methoxy	31±12	17
4h	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2(5-tetrazolyl); 4-methoxy	29±3	0
4i	Et	CH_2COOH	3,4-Methylenedioxy	2-$O(CH_2)_3COOH$; 4-methoxy	22±7.5	37
4j	Et	CH_2COOH	3,4-Methylenedioxy	2-$OCH_2C(CH_3)_2COOH$; 4-methoxy	68±17	1
4k	Et	CH_2COOH	3,4-Methylenedioxy	2-(2-Hydroxyethoxy); 4-methoxy	>10,000	6
4l	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3-methoxy	85±13	12
4m	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3,4-dimethoxy	6.1±1.9	2
4n	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3,4,5-trimethoxy	310±41	1
4o	Et	CH_2COOH	3,4-Methylenedioxy	3,4,5-Trimethoxy	66±16	17
4p	*n*-Pr	CH_2COOH	2-OCH_2COOH; 4-methoxy	4-Methoxy	>10,000	0

[a]Mean ± SEM, $n = 3$.
[b]not determined.

Table 2. Inhibition of [^{125}I]ET-1 binding to ET_A and ET_B receptors by *cis* 1,3-disubstituted isoindoline analogues **5**



Compd	R^1	X	R^2	R^3	ET_A-IC_{50} (nM)[a]	ET_B–%Inh @ 1 μM
5a	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 4-methoxy	4.1±1.9	52
5b	Et	CH_2COOH	3,4-Methylenedioxy	4-Methoxy	95±8	23
5c	Et	CH_2COOH	3,4-Methylenedioxy	2-Hydroxy; 4-methoxy	58±13	13
5d	Et	CH_2COOH	3,4-Methylenedioxy	2-$OCH_2C(CH_3)_2COOH$; 4-methoxy	14±2.8	34
5e	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2(5-tetrazolyl); 4-methoxy	>10,000	37
5f	Et	COCOOH	3,4-Methylenedioxy	2-OCH_2COOH; 4-methoxy	>10,000	0
5g	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3-methoxy	110±16	31
5h	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3,4-dimethoxy	96±5.5	27
5i	Et	CH_2COOH	3,4-Methylenedioxy	2-OCH_2COOH; 3,4,5-trimethoxy	330±37	10
5j	Et	CH_2COOH	3,4-Methylenedioxy	3,4,5-Trimethoxy	1100±120	15
5k	Et	5-Tetrazolyl	3,4-Methylenedioxy	3,4,5-Trimethoxy	>10,000	0
5l	*n*-Pr	CH_2COOH	2-OCH_2COOH; 4-methoxy	4-Methoxy	>10,000	0

[a]Mean±SEM, $n=3$.

slightly improved activity compared to the parent 4-methoxyphenyl compound **4b**. However, addition of a second acid through the trimethoxyphenyl group (i.e., compound **4n**) did not enhance the potency analogously to **4a**. In fact, **4n** was about 5 times less active than **4o**. Interestingly, removal of a methoxy group from the 5-position of the trimethoxyphenyl substituent in analogue **4n** afforded a derivative, **4m** ($ET_A-IC_{50}=6.1$ nM), with comparable activity to **4a**. Similar trend was not observed in the *cis* series of isoindolines, compounds **5j**, **5i**, and **5h**. However, with both isomers moving the 4-methoxy group in compounds **4a** and **5a** to the 3-position of the phenyl ring, derivatives **4l** ($ET_A-IC_{50}=85$ nM) and **5g** ($ET_A-IC_{50}=110$ nM), led to a significant loss of binding affinity. Although the importance of the alkoxy substituent at the isoindoline 5-position has not been thoroughly studied it appears from the existing data that an ethoxy group at this binding site is close to an optimal size. While the corresponding *n*-propoxy analogue **4d** ($ET_A-IC_{50}=120$ nM) retains the activity of the ethoxy compound **4b**, the methoxy derivative **4e** ($ET_A-IC_{50}=3300$ nM) is considerably less potent. Furthermore, when the side-chain carboxylic acid moiety is moved from the aryl substituent at the isoindoline 3-position to the aryl group at the 1-position, compounds **4p** and **5l**, the binding activity in both *trans* and *cis* series is totally abolished (ET_A-IC_{50}'s > 10 μM).

In summary, replacement of the indane ring in compound **1** with an isoindoline gave rise to a new class of ET receptor antagonists. Contrary to compound **1**, isoindoline analogues **4** and **5** are selective for the ET_A receptor subtype. Furthermore, the data presented here clearly demonstrate the necessity of two appropriately positioned carboxylic acid groups to achieve low nanomolar binding affinity in the isoindoline class of compounds.

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20. All compounds were characterized by elemental analysis, ^{1}H NMR, IR, and DCI-MS.
21. Chinese hamster ovary cells expressing human ET_A (CHO-ET_A) or ET_B (CHO-ET_B) receptors were cultured in D-MEM/F12 medium (GibcoBRL, Grand Island, NY) containing 10% fetal bovine serum, 1× antibiotic/antimycotic (GibcoBRL), and 0.3 mg/mL geneticin. Cells were harvested by scraping, pelleted by centrifugation, and homogenized in a buffer containing 1 mM EDTA and 10 mM Tris, pH 8.0. The cell debris was removed by brief centrifugation, and the supernatant was centrifuged again at 50,000×*g* for 10 min. The resulting pellet was resuspended in Hanks' balanced salt solution at a protein concentration of 0.2 mg/mL and stored in aliquots at −80 °C. Competition binding was carried out by incubating 0.2–1.0 μg of membrane protein from CHO-ET_A or 8 μg from CHO-ET_B cells with 12,000 cpm [^{125}I]ET-1 (New England Nuclear, Boston, MA) with or without the competing compound for 2 h at 37 °C in a buffer containing 0.2 mg/mL bovine serum albumin, 0.002% Triton X-100, 0.02% NaN_3, and 50 mM Tris, pH 7.0. The reaction was terminated by filtration through a cell harvester (Brandel, Gaitherburg, MD) using GF/C filters. The radioactivities on the filters were counted in a gamma counter. Triplicate samples were assayed in each experiment, and the full dose–response curve for each competing compound was performed at least three times. The IC_{50} values were determined by a nonlinear least-squares curve-fitting program.
22. The IC_{50} values for the ET_B receptor binding were not determined for compounds with less than 50% inhibition at 1 μM concentration.
23. The pK_B values were measured following a protocol described by Shetty, S. S.; DelGrande, D.; Savage, P.; Ksander, G. M.; Jeng, A. Y. *J. Cardiovasc. Pharmacol.* **1995**, *26*, S310.